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April 17, 1998

Dr. C. W. Jameson
National Toxicology Program
Report on Carcinogens
MD EC-14
P.O. Box 12233
Research Triangle Park, NC 27709

Re: Comments on Mixtures and Exposure Circumstances Proposed for Listing in the Report on Carcinogens, Ninth Edition

Dear Dr. Jameson:

The Oxygenated Fuels Association, Inc. (OFA) appreciates this opportunity to comment on the National Toxicology Program (NTP) proposal for including methyl-*tertiary*-butyl ether (MTBE) in the Ninth Edition of its Report on Carcinogens (*Federal Register* notice Vol. 63, No. 22, page 5565, February 3, 1998).

The OFA recommends that MTBE not be listed in the report because (1) no epidemiologic evidence exists to indicate or suggest that MTBE might cause cancer, and (2) the evidence from the systematic evaluation of possible mechanisms of carcinogenic action in laboratory animals are compelling in indicating that MTBE acts "through mechanisms that do not operate in humans, and therefore, not reasonably be anticipated to cause cancer in humans." More detailed scientific comments and discussions that support OFA's conclusions are provided in the enclosed document.

The Department of Health and Human Services, National Toxicology Program's annual Report on Carcinogens (Ninth Edition) is a congressionally-mandated list of known and reasonably anticipated human carcinogens, and its release is an important annual public event. Thus, confirming or even suggesting that a substance is a human carcinogen is not to be undertaken lightly.[1] At the very least, the consideration of MTBE should be delayed until (1) MTBE is nominated following the procedures provided by the NTP, (2) data clearly and convincingly satisfy the NTP listing criteria, (3) any uncertainties about interpretation that can be promptly resolved have been resolved, and (4) agency and interagency reviews have been concluded that might assist the NTP in deciding whether to consider listing the substance.

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[1] The court in *Synthetic Organic Chemical Manufacturers Association (SOCMA) v. Secretary, Department of Health and Human Services*, 720 F. Supp. 1244 (W.D. La. 1989), held that additions to the Report on Carcinogens were judicially reviewable, even though such listings do not create direct obligations or have enforcement effect, because of the "moral suasion" of the Report. At 1250. See also *Flue-Cured Tobacco Cooperative Stabilization Corp. v. U.S. Environmental Protection Agency*, 857 F. Supp. 1137 (M.D. N.C. 1994) "This court agrees that 'it takes little knowledge of the goings-on about us to be aware that "moral suasion" is a considerably potent force in our society...'." At 1143 (citing SOCMA).

Under the policies provided by the NTP, substances must be nominated for listing.[2] The EPA Office of Research and Development (ORD) is reported to have nominated MTBE for listing as a "reasonably anticipated human carcinogen," but the NTP has no written record of the nomination. When copies of the nomination and supporting rationale and documentation were requested from EPA, in order for stakeholders such as OFA to be better equipped to comment on the proposal, Dr. J. Parker of the EPA ORD provided Dr. C. W. Jameson of the NTP a one-page email note, confirming on April 8, 1998, that she intended to make the nomination, and providing a conclusory one-paragraph explanation of her basis for nominating MTBE. Supporting analysis and documentation were neither referenced nor provided.

Based on our review of the one-page after-the-fact email note, OFA is quite confident that it would be premature, and, to paraphrase the language of the statute, "unreasonable" to include MTBE in the annual list at this time. OFA believes the matter should be returned to the EPA (although it is not clear that EPA itself formally supports Dr. Parker's position) with the suggestion that EPA undertake further consideration and permit the completion of several reviews of MTBE's potential hazard characterization that are actively under way at this time.

NTP procedures specifically provide for the outcome recommended by OFA. The procedures state "(I)f it is determined that the petition contains insufficient information to warrant consideration by the NTP, it may be returned to the original petitioner who may be invited to resubmit the petition with additional justification, which may include new data, exposure information, etc." Dr. Parker's email note cannot substitute for the absence of a petition to support the NTP's notice in the February 3, 1998 *Federal Register* asking for comment on MTBE. Even if the email note were to be viewed as an after-the-fact "petition," it provided an inadequate basis for proceeding further with consideration of MTBE at this time.

NTP procedures provide that it may initiate an independent search of the literature and prepare a draft review document for a substance that has been properly nominated. MTBE has not been properly nominated. Even if MTBE had been properly nominated, instead of conducting an independent review at this time, adding to the number of reviews of MTBE now under way, OFA suggests that NTP delay further consideration of MTBE until the EPA Office of Water further addresses MTBE potential carcinogenicity hazard, and until completion of (1) the mechanistic studies being conducted by the Chemical Industry Institute of Toxicology (CIIT) (which includes an external advisory committee that includes prominent federal agency risk scientists), (2) an assessment by scientists at the University of California under a state statutory program to examine potential MTBE health risks, and (3) a major oral animal study that will be started shortly under the auspices of the Oxygenated Fuels Association.

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[2] February, 1998; see also 63 Fed. Reg. 5565, 66, February 3, 1998.

A major conclusion of the two-year federal Interagency Assessment of Oxygenated Fuels performed by the National Science and Technology Council in the Executive Office of the President under the direction of the President's Science Advisor stated: "Estimates of cancer potency derived from MTBE animal studies as well as estimates of human exposure to MTBE have large uncertainties and caution is required in their use."[3] Recently, in December 1997, the EPA's Office of Water drew back from releasing a final health-based drinking water advisory that included quantitative estimates of the health risks of MTBE, including cancer. Instead, the EPA's provisional consumer acceptability advisory focused upon MTBE's odor and taste characteristics in water, which are detected at low levels that they in effect provide an "early warning" that abundantly protects the public from exposures to MTBE at levels that might pose an increased health risk. The Office of Water made it clear in the provisional advisory that "there are many uncertainties and limitation associated with the toxicity database for this chemical (MTBE)."[4] Specifically, the Office of Water stated that "limitations of the reported studies do not allow confident estimates of the degree of risk MTBE may pose to humans from low-level drinking water contamination." The Office of Water pointed out that toxicokinetics models are limited in helping to extrapolate from inhalation data to risk from drinking water intake, a subject which is specifically being addressed by the ongoing CIIT research and the OFA-sponsored subchronic oral animal study. The Office of Water concluded, regarding its own drinking water advisory, that "additional research is needed to resolve these issues before a more complete health advisory can be issued." The same could be said in support against NTP listing of MTBE as reasonably anticipated to be a human carcinogen, especially since many uncertainties and answers to key cancer risk questions can be more fully addressed in the near future.

Despite a large volume of work on MTBE's potential carcinogenicity, and evidence that MTBE is carcinogenic in rats and mice, the chronic studies performed to date raise a number of significant issues about alternative explanations for the animal tumors, study designs, pathology interpretations, and data reporting. These issues are being actively addressed in various ongoing studies and reviews. An important issue, given the source of the nomination of MTBE, is the EPA's Office of Water, which has stated that it has put off addressing MTBE cancer risk potential and plans to publish another advisory that includes health-based evaluations for MTBE at a later date. Thus, the EPA has not yet decided itself how to address the potential for MTBE to cause human cancer. The NTP would be well advised to return this matter to the nominating office with the suggestion that it complete ongoing review(s) on MTBE.

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[3] Executive Summary, page vii, June, 1997.

[4] U.S. EPA Provisional Advisory, page ii, December, 1997.

Another important research activity is taking place at the CIIT. This research addresses the critical determinants of rodent toxicity testing which may be used to estimate the potential hazard and risk posed to humans by MTBE exposures. The research is investigating the mechanisms related to high-dose MTBE exposures in animal testing and developing a physiologically-based dosimetry model for MTBE and its metabolites to use in low-dose, species-to-species extrapolation of health risk potential. It should be stressed that the CIIT research is being carefully reviewed by an outside advisory committee that has the purpose of guiding the direction and technical design of the program, and interpretation and meaning of the experimental results generated. The advisory committee is composed of representatives from the U.S. Environmental Protection Agency, National Institute of Environmental Health Sciences, Pacific Northwest National Laboratory, Wisconsin Department of Health & Social Sciences, and several industry groups.

A third activity of importance to the NTP consideration is the California State Legislature's recently mandated scientific review of MTBE to be conducted by four different centers of the University of California system. The NTP would be well advised to await the outcomes of this extensive review.

The fourth activity of significant importance is that OFA has just undertaken to conduct a well-designed animal study to investigate further the relevance and significance of previous chronic health testing of MTBE. This 90-day, oral route of exposure study will examine whether systemic organ toxicity is similar to inhalation exposure (and thereby support route-to-route extrapolations), and obtain tissue-specific mechanistic data. Previously conducted oral exposure testing (using gavage administration with vegetable oil vehicle) have significant and serious technical problems and uncertainty in data presentation and interpretation for human hazard potential. The OFA study being undertaken is designed to address issues regarding hazard characterization and risk potential for oral (i.e., drinking water) exposures to MTBE.

In conclusion, as presented in this letter and the enclosed comments, OFA believes not only that it would be premature at this time for the NTP to list MTBE as a substance reasonably anticipated to be a human carcinogen, but also in direct violation of published NTP procedures for nomination and consideration of MTBE for listing. Numerous federal agencies and scientists, university scientists, and other qualified experts have identified serious issues with studies and risk analyses that indicate MTBE may have potential for human cancer hazard, and those issues are under active investigation and review. Such an approach seems the better course in light of the "moral suasion" and publicity that surrounds the release of the NTP report. The resolution of these problems would permit a judgement whether MTBE should be incorporated to the NTP list as reasonably anticipated to be a human carcinogen. Further, it would be

superfluous and inefficient for NTP to launch yet another review internally that would essentially duplicate the already ongoing reviews taking place by the EPA and other entities.

The OFA welcomes the chance to meet with the NTP staff at the earliest possible convenience to discuss our comments if necessary.

If there are any questions about our submission and comments, please feel free to contact me at 703-841-7100.

Sincerely,

John Kneiss
Director, Health Sciences &
Product Stewardship

Enclosure

Comments
Concerning a Proposed Listing
in the NTP Biennial Report on Carcinogens:

Methyl-*tertiary*-Butyl Ether (MTBE)

Submitted to
The U.S. National Toxicology Program
Attn: Dr. C. W. Jameson
79 Alexander Drive, Building 4401, Room 3127
PO Box 12233, MD WC-05
Research Triangle Park, North Carolina 27709

Submitted by
The Oxygenated Fuels Association
Arlington, Virginia

20 March 1998

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Methyl-*tertiary*-Butyl Ether (MTBE)

1 Introduction

The Oxygenated Fuels Association (OFA) provides these comments to the National Toxicology Program (NTP) in response to the Program's proposal to list methyl-*tertiary*-butyl ether (MTBE) in the Ninth Edition of its Report on Carcinogens. OFA recommends that MTBE not be listed in this report because (1) no epidemiologic evidence exists to indicate or suggest that MTBE might cause cancer, and (2) the evidence from the systematic evaluation of possible mechanisms of carcinogenic action in laboratory animals are compelling in indicating that MTBE acts "through mechanisms that do not operate in humans, and, therefore, not reasonably be anticipated to cause cancer in humans."

The Oxygenated Fuels Association, Inc. is an international trade association, incorporated in 1983, to advance the use of and knowledge about oxygenated fuel additives that improve the combustion performance of gasoline, thereby reducing automobile pollution.

On 3 February 1998, NTP announced "its intent to review additional substances, mixtures, and exposure circumstances for possible listing or delisting (removing) from the Report on Carcinogens, Ninth Edition" (Federal Register 1998). In accordance with a Congressional mandate, this Report is to list two categories of substances: (1) "known human carcinogens," and (2) "reasonably anticipated human carcinogens." The Federal Register announcement goes on to describe the review process for listing or delisting of substances. Included among the substances for consideration for listing is MTBE (CAS # 1634-04-4), a major additive in automotive fuels in the U.S. that concentrations of toxicants in auto exhaust and ambient air.

The comments presented below and the data in the attached published papers are intended to inform NTP's deliberations about whether MTBE should be included or excluded from the next edition of the Report. OFA's comments are organized into a summary of NTP's criteria for either listing or delisting substances in its Reports (Section 2), a critical evaluation of the data used to elucidate mechanisms of carcinogenic action in laboratory rodents and

their relevance to humans (Section 3), a summary and conclusions of the findings (Section 4), and the citations (Section 5).

2 Criteria for Listing and Delisting

NTP has described a set of criteria by which to decide whether a substance is to be included in either of the two listing categories ("known human carcinogen" and "reasonably anticipated to be a human carcinogen") in the Biennial Report on Carcinogens (NTP, 1996).

According to these criteria, to be included as a *known human carcinogen*:

> "sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance, or mixture and human cancer" is needed.

Correspondingly, to be included as a *reasonably anticipated to be human carcinogen*:

> "sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors:
> (1) in multiple species or at multiple tissue sites;
> (2) by multiple routes of exposure; or
> (3) to an unusual degree with regard to incidence, site, or type of tumor or age at onset;

or

> there is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well defined structurally-related class of substances whose members are listed in a previous Annual or Biennial Report on Carcinogens as either a known to be human carcinogen or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans."

NTP acknowledges that applying these criteria requires "scientific judgment, with consideration given to all relevant information." Such relevant information includes, according to NTP, dose response metabolism, pharmacokinetics, sensitive sub-populations, genetic effects, and other data relating to mechanism of action or factors that may be unique

to a given substance. NTP specifically illustrated these considerations by defining the case in which evidence for carcinogenicity exists "but there were compelling data indicating that the agent acts through mechanisms which do not operate in humans and would, therefore, not reasonably be anticipated to cause cancer in humans."

These criteria will be applied to wide-ranging toxicologic data on MTBE (Section 4).

3 Evaluation of Relevant Data

Although MTBE has been used as a therapeutic agent in humans (see Bibliography: Citations Related to Gallstone Dissolution by MTBE, below) and occupational exposures to MTBE have occurred for nearly two decades because of its high volatility, no studies of humans have indicated or suggested that MTBE is directly or indirectly associated with cancer in humans. Therefore,

No data or justification exists to list MTBE in NTP's Report as a "known to be carcinogen."

Three studies have been performed in rodents to evaluate the carcinogenic potential of MTBE, two by inhalation (Bird *et al.* 1997) and one by gavage (Belpoggi *et al.* 1995).

The carcinogenic potential of **inhaled MTBE** was investigated in Fischer 344 **rats** (Bird *et al.* 1997). Fifty animals/sex/species/dose were administered doses of 0, 400, 3000, and 8000 ppm MTBE vapor for six hours per day, five days per week, for 24 months. The traditional measurements, including histopathology, for chronic toxicity studies were performed. Results at the highest dose tested indicated CNS depression, body and organ weight changes, increased mortality, and decreased survival rate. High premature mortality and organ/body weight changes were observed in the high dose group, suggesting that the maximum tolerated dose is likely to have been exceeded. Increased liver and kidney weights in both sexes were observed at the 3000 and 8000 ppm dose levels; however, no accompanying histopathological changes were observed in the liver. Microscopic examination confirmed a dose-dependant increase in progressive kidney nephropathy in males at all dose levels and to a lesser extent in mid- and high-dose females typical of that observed for aging rats but more severe in MTBE-treated animals. Based on histological examination, a statistically significant increase in renal tubular cell adenomas and carcinomas was reported in males exposed to 3000 or 8000 ppm MTBE. The authors hypothesized that this increased tumors rate may be the result of the accumulation of a species-specific protein within the male rat kidney. In addition, an increased incidence in interstitial cell adenomas of the testes was greater in males exposed to 3000 and 8000 ppm, due to an artifact of an unusually low control incidence and not to the test agent.

The carcinogenic potential of **inhaled MTBE** was also investigated in CD-1 **mice** (Bird *et al.* 1997). Fifty animals/sex/species/dose were administered doses of 0, 400, 3000, and 8000 ppm MTBE vapor for six hours per day, five days per week, for 18 months. The traditional measurements, including histopathology, for chronic toxicity studies were performed. Results indicated CNS depression, body and organ weight changes, increased mortality, and

decreased survival rate. The high mortality and organ/body weight changes observed in the high dose group indicated that the maximum tolerated dose is likely to have been exceeded. Increased corticosterone levels were observed in both sexes at the high dose level however this finding was only statistically significant for male mice. Increased liver (3000 and 8000 ppm females) and kidney weights (all males; 8000 ppm females) were also observed. During histological examination, a decreased incidence of cystic endometrial cell hyperplasia was observed in 3000 and 8000 ppm female mice. Hepatocellular hypertrophy was increased in 3000 ppm males and both sexes at 8000 ppm. A statistically significant increase in hepatocellular adenomas was observed in females at 8000 ppm. The authors stated that, because MTBE has been demonstrated to be non-genotoxic and non-active in an *in vivo* micronucleus and hepatocyte unscheduled DNA synthesis assay, these female mice liver tumors may have resulted from the promotion by MTBE of spontaneously initiated liver cells.

MTBE, administered by **gavage**, was tested for possible carcinogenicity in **rats** (Belpoggi *et al.* 1995). Eight-week old Sprague-Dawley rats (60 animals/sex/dose) were administered doses of 0, 250, and 1,000 mg/kg-bw MTBE, dissolved in olive oil, via gavage four days a week for 104 weeks. At death, animals were submitted to systemic necropsy, and histopathology was performed on all organs and tissues. Results indicated no differences in water and feed consumption, mean body weight gain, behavioral changes, and nononcological changes. Survival rates were higher among high-dose males and showed a dose-dependent decrease among females after 16 weeks of treatment. The authors reported a statistically significant increase in testicular Leydig cell tumors at the high dose and a dose-dependent increase in lymphomas and leukemias among females. Lastly, the authors reported an increase incidence in dysplastic proliferation of lymphoreticular tissue and mammary fibromas and fibroadenomas in females, and speculated that the doses of MTBE had been sufficiently high to increase these incidences as well as premature mortality.

A considerable body of relevant and compelling evidence exists to indicate that MTBE would **not** reasonably be anticipated to cause cancer in humans, although some have suggested that MTBE might be listed as "reasonably anticipated to be human carcinogen." The data that impact on the interpretation of the significance of the carcinogenicity findings are described below.

1. MTBE is not genotoxic in numerous assays, and, therefore, is not likely to be a genotoxic carcinogen.

Two generic classes of mechanisms of carcinogenesis are used by numerous scientists and decision makers as a means of distinguishing expected differences in the risks to humans and

to orient research initiatives. Genotoxic mechanisms of carcinogenesis are generally treated as having a linear dose-response with the inference that a finite risk exists at all doses. By contrast, non-genotoxic mechanisms (*e.g.*, increased cellular proliferation), are traditionally associated with non-linear dose response and are consistent with biological thresholds, implying that risk is unlikely to exist below the threshold values.

MTBE has been tested in numerous *in vivo* and *in vitro* assays, and found to produce no mutations or other genotoxic effects.

Four *in vivo* genotoxicity assays were conducted to explore the potential mutagenicity of MTBE (McKee *et al.*, 1995). These tests included the *Drosophila* sex-linked-recessive-lethal test, the rat bone marrow cytogenetics test, the mouse bone marrow micronucleus test, and the *in vivo-in vitro* hepatocyte unscheduled DNA synthesis test in mice. The authors reported that the results of all tests were negative, suggesting that MTBE has a low probability *in vivo* for mutagenic activity. The authors concluded that the rodent tumors attributed to near-lethal doses of inhaled MTBE and reported by Bird *et al.* (1997) were the result of non-genotoxic mechanism(s).

MTBE has also been tested in three *in vitro* assays. Cinelli *et al.* (1992) examined the potential mutagenic activity of MTBE by assessing the induction of gene mutation in *Salmonella typhimutium* (with and without metabolic activation; 5 dose levels) and in established Chinese hamster V79 fibroblasts (with and without metabolic activation; 5 dose levels), and the induction of unscheduled DNA synthesis in primary rat hepatocytes in culture. Neither toxicity nor induction of reverse mutation was observed at any dose level either with or without metabolic activation in the *Salmonella* test. Gene mutation was not induced in Chinese hamster V79 cells, even though, in the presence of S9 metabolism, cell survival declined steeply between concentrations of 1.25 and 10 mg/ml. Unscheduled DNA synthesis was not induced in primary rat hepatocytes. Also, MTBE did not increase the mean net grain count of five grains per nucleus at treatment levels between 1 and 10 mg/ml.

One study reported MTBE-induced genotoxicity in an S9-activated mouse lymphoma assay (McGregor *et al.* 1988). That result has been shown to be mis-attributed to MTBE, and attributed to unusually high concentrations (i.e., μM/mL) of formaldehyde, a potential mutagen, in the S9-activated mouse lymphoma assay (Garnier *et al.* 1993). Although formaldehyde is a minor and transient (half-life estimated as approximately 1.5 minutes) metabolite of MTBE *in vivo*, it is unlikely to be present at similar cellular concentrations when MTBE is inhaled or ingested by humans at reported ambient levels. The findings of Cassanova and Heck (1997) (described more fully in Conclusion 3) clearly demonstrated that, although formaldehyde is a minor metabolite of MTBE in rodents, it is unlikely to be

a mutagen *in vivo* as a result of ambient exposures to MTBE because (1) the concentrations of formaldehyde from MTBE were very small; and (2) the absence of concentration-dependent DNA-protein cross-links and of RNA-formaldehyde adducts—each a biomarker of biologically reactive formaldehyde—such that the rate of formaldehyde production is sufficiently slow to preclude reaching saturation of formaldehyde and to assure that formaldehyde from MTBE is detoxified before it might react with genetic macromolecules. Therefore, the metabolism of MTBE to formaldehyde is not a critical component of MTBE's carcinogenic mechanism in mice and most likely rats.

Taken together, these studies indicate that MTBE is not genotoxic, and, therefore, is not likely to be a genotoxic carcinogen. This was the conclusion of an international organization (ECETOC, 1997): "The weight of evidence suggests that MTBE is not genotoxic." As will be noted below, MTBE's mechanisms of carcinogenicity are derived predominantly from doses sufficient to cause substantial damage and from biochemical changes unlikely to occur in humans exposed to ambient levels of MTBE.

2. Compelling evidence exists to indicate that the male rat kidney tumors are likely to result from secondary, non-genotoxic tissue damage caused by otherwise highly toxic doses of MTBE—doses unlikely to be experienced by humans; and this mechanism is unlikely to exist in humans.

MTBE at inhaled toxic doses induced renal tumors only in male rats, and not in male or female mice similarly exposed. The relevance of these responses to humans is seriously questioned. Based on histopathological observations and pattern of tumor induction, this gender- and species-specific response was hypothesized to result from a multi-step process, the first of which was the deposition of protein droplets unique to the male rat kidney and to the substantial magnitude of the doses presented to the animals.

A series of mode of action studies have addressed the likelihood that protein deposition was an obligatory first step in tumorigenesis, and that one protein in particular, α_{2u}-globulin, was dominant in the precipitate in stimulating the pathogenesis process in kidney cells. This particular protein has been shown through extensive research to be generated solely in rats and not in humans, because of major biochemical differences between these two species. In the absence of this protein precipitate, it can be reasoned, no kidney injury will occur, and hence the stimulus for tumorigenesis is absent. Furthermore, in the presence of large amounts of α_{2u}-globulin in the male rate kidney of MTBE-treated animals, it can be further argued, the induction of tumors in humans would be most unlikely to occur because humans do not have the biochemical requisites to form this protein in their kidneys, regardless of the

dose of MTBE or other substances (*e.g.*, trimethylpentane) known to elicit this protein only in male rate kidneys and in no human tissues.

The Chemical Industry Institute of Toxicology (CIIT) has undertaken a major research program to explore mechanism(s) by which MTBE causes this tumor in rats with the goal of reducing the uncertainty in assessing human risk from low-level exposures to MTBE over a lifetime (Borghoff *et al.* 1996). The CIIT research program addresses several issues relevant to the long-term cancer responses observed in rats and mice exposed repeatedly to MTBE.

Initially, Borghoff *et al.* (1990) stated the plausibility of such biochemical mechanisms and pathobiology of nephropathy in male rats as it relates to MTBE. Drawing from observations of some other kidney carcinogens, the authors hypothesized that the complex of MTBE bound to α_{2u}-globulin would lead to a less digestible chemical-protein complex. Because of the decreased hydrolysis of this chemical-protein complex, an excessive accumulation of chemical-protein droplets would occur in lysosomes of proximal tubule epithelial cells in the P_2 segment of the nephron. This accumulation of chemical-protein droplets in these proximal tubules would cause an increase in the size and number of secondary lysosomes. Lysosome overload would result, and progress to degeneration and necrosis of individual cells lining the P_2 segment. This tubular epithelial cell necrosis and exfoliation would lead to restorative cell proliferation in the P_2 segment as a compensatory response to the increased cell loss from toxicity. This restorative cell replication would be the proximate cause of renal carcinogenesis in male rats. The authors noted that, unlike mature male rats, immature male and female rats do not produce α_{2u}-globulin in their livers and, therefore, are not expected to, and do not, develop α_{2u}-globulin nephropathy or renal cancer. The authors also noted that humans do not produce α_{2u}-globulin. Therefore, because this syndrome appears to be dependent on the presence of α_{2u}-globulin, logically, humans would not be at any risk of developing α_{2u}-globulin nephropathy or renal cancer when exposed to chemicals such as MTBE.

Results of this line of investigation are reported in several scientific publications. Because MTBE and its metabolite t-butyl alcohol both cause renal tumors in chronically exposed male rats, knowledge of the kinetic behavior of MTBE and t-butyl alcohol in rats and its comparison to the kinetics of these chemicals in humans aid in assessing human risk. The objective of a study by Borghoff *et al.* (1995) was to develop a physiologically based pharmacokinetic model for MTBE and t-butyl alcohol in rats that would form the basis for a human model. Physiological parameters such as blood flows, tissue volumes, and alveolar ventilation were obtained from the literature. Chemical-specific parameters such as the solubility of MTBE and t-butyl alcohol in blood and selected tissues and metabolic rate

constants to describe whole-body metabolism of MTBE in rats were measured using vial equilibration and gas uptake techniques, respectively. MTBE metabolism was described in the model as occurring through two saturable pathways. The model was able to predict gas uptake data (100 to 2000 ppm starting concentrations) and levels of MTBE in blood of rats exposed to MTBE by inhalation (400 and 8000 ppm, 6 hr), *i.v.* (40 mg/kg), and oral (40 or 400 mg/kg) administration. Two different models to describe the dosimetry of t-butyl alcohol in the rat were tested for their ability to predict t-butyl alcohol blood levels after MTBE exposure. *T*-butyl alcohol blood levels were predicted best at low MTBE exposure concentrations using a two-compartment model. The toxicokinetics of *t*-butyl alcohol appear to be far more complex than those of MTBE. With a quantitative description of the important determinants of MTBE and *t*-butyl alcohol dosimetry understood, a better assessment of the potential toxic and cancer risk for humans exposed to MTBE can be made.

In the process of measuring chemical specific parameters necessary to develop a quantitative dosimetry model of MTBE in rats, the uptake of MTBE was found to be 5.5 times greater in male than in female F-344 rat kidney homogenate (Poet and Borghoff, 1997). The objectives of this study were to characterize the factor(s) that influences the high uptake of MTBE into male rat kidney *in vitro* and to develop a system to evaluate the interaction of MTBE with the male rat-specific protein, α_{2u}-globulin. The uptake of MTBE in male, but not female, rat kidney homogenate was found to be dependent on protein and chemical concentrations. When [^{14}C]MTBE was incubated with male rat kidney homogenate, radioactivity coeluted with the total protein fraction on a gel filtration column. An interaction between [^{14}C]MTBE and male rat kidney proteins was not found under conditions of dialysis or anion exchange chromatography. A two-compartment vial equilibration model was used to assess the interaction between MTBE and α_{2u}-globulin. Using this system, the dissociation constant for MTBE and α_{2u}-globulin was estimated to be 2.15 x 10^{-4} M, which is in the range of other chemicals known to bind to α_{2u}-globulin and cause α_{2u}-globulin-mediated nephropathy. *d*-Limonene oxide was used to validate this two-compartment vial equilibration system. These findings illustrate a technique useful in estimating the dissociation constant for a volatile chemical and a protein, as well as explain the process that contributes to the uptake of MTBE into male rat kidney homogenate *in vitro*. A description of the weak interaction between MTBE and α_{2u}-globulin is used to refine a physiologically based toxicokinetic model to describe the target tissue (kidney) concentrations of MTBE.

The objective of a study by Prescott-Mathews *et al.* (1997) was to determine if MTBE induces an α_{2u}-globulin nephropathy and renal cell proliferation in male F-344 rats. Male and female F-344 rats were exposed to MTBE vapors of 0, 413, 1515, or 3013 ppm for six hours/day for 10 consecutive days. Significant proximal tubule necrosis and protein droplet accumulation were observed in kidneys from male rats exposed to 1515 and 3013 ppm

MTBE. Significantly greater labeling indices were observed in all groups of MTBE-exposed male rats. α_{2u}-Globulin immunoreactivity was present in and confined to protein droplets in male rat kidney. A mild dose-related increase in α_{2u}-globulin concentration in the kidney, as measured by an enzyme-linked immunosorbent assay, was observed in male rats exposed to MTBE, with a statistically significant increase in α_{2u}-globulin concentration in male rats exposed to 3013 ppm MTBE. A strong positive correlation (r = 0.994) was found with exposure concentration between cell proliferation and α_{2u}-globulin concentration in male rat kidney. No significant differences were observed in female rats for any of these responses. Further analysis of kidney cytosol failed to demonstrate the accumulation of any protein besides α_{2u}-globulin in MTBE-exposed male rat kidney. These findings demonstrate that MTBE causes a mild induction of α_{2u}-globulin nephropathy and enhanced renal cell proliferation in male, but not female, F-344 rats, suggesting a substantive role for α_{2u}-globulin nephropathy in renal tumorigenesis.

CIIT has concluded, based on MTBE kidney responses and other data available on MTBE, that MTBE appears to be a mild inducer of α_{2u}-globulin nephropathy in the male rat and that other proteins do not appear to be involved in the accumulation of protein droplets. In addition, CIIT concluded that kidney cancer occurring in MTBE-exposed male rats should not be used in the hazard identification of MTBE as it pertains to humans (Borghoff *et al.*, 1996).

These data suggest that inhaled high doses of MTBE lead to concentrations of MTBE and/or TBA in the kidneys that are sufficiently high to precipitate α_{2u}-globulin, and they indicate that the MTBE binds with the α_{2u}-globulin to produce a highly insoluble protein precipitate that is capable of causing tissue damage that might eventuate into renal tumors. According to Bird *et al.* (1997), these findings are reinforced by observations from the NTP bioassay of TBA (NTP, 1995) in which TBA-treated male rats showed classical signs of α_{2u}-globulin nephropathy including increased protein droplets in renal tubular epithelial cell. These data are consistent with the hypothesis that MTBE induced kidney tumors in male rats is mediated by a mechanism that is present in male rats only and is not present in human kidneys.

Correspondingly, a committee of the National Academy of Sciences (NRC 1996) has concluded "that the male rat kidney-tumor data probably should not be used for (estimating the cancer potency of MTBE) in light of the new information on its probable causation, *i.e.* α_{2u}-globulin nephropathy, which is thought to be unique to the male rat and **not relevant to humans** (emphasis added)."

Therefore, compelling evidence exists to indicate that MTBE-induced male rat kidney tumors are likely to result from secondary, non-genotoxic tissue damage caused by otherwise highly toxic doses of MTBE.

3. Mounting evidence strongly suggests that the female mouse liver tumors are the result of high non-genotoxic doses of MTBE interfering with the tumor suppressor mechanisms of estrogen in female mouse liver—mechanisms that are not present in human liver.

MTBE at inhaled toxic doses induced liver tumors only in female mice, and not in male mice or in male or female rats similarly exposed. The relevance of these responses to humans is seriously questioned. Based on histopathological observations and pattern of tumor induction, this gender- and species-specific response was hypothesized to result from a multi-step process, the first of which was secondary mechanism(s) of tumor induction and, second, to the high concentration of the doses presented to the animals.

A series of mode of action studies have addressed the likelihood that MTBE might induce liver tumors at inhaled toxic doses only in female mice, and not in male or female rats similarly exposed. These mode of action studies have addressed the likelihood that MTBE might induce liver tumors through a progression of three hypotheses that were systematically tested. Two of the hypotheses have been subsequently discounted, and the most recent to be raised has shown the greatest promise of defining the extent to which this tumor response is species- and gender-specific and not relevant to humans.

Based on previous studies conducted on unleaded gasoline and MTBE which resulted in the increased incidence of liver tumors selectively in female mice, Moser *et al.* (1996) speculated that MTBE would have hepatic tumor-promoting activity in the same initiation-promotion model system in which unleaded gasoline was a hepatic tumor promoter. To investigate this hypothesis, 12-day old female $B6C3F_1$ mice were initiated with a single *i.p.* injection of the mutagen *N*-nitrosodiethylamine (5 mg *N*-nitrosodiethylamine/kg, 7.1 ml/kg-bw) or saline. Beginning at eight weeks of age, mice were exposed to 0 ppm or the hepatocarcinogenic dose of approximately 8000 ppm MTBE. After subchronic exposure of 16 or 32 weeks, MTBE significantly increased liver weight and hepatic microsomal cytochrome P450 activity without hepatotoxicity or an increase in non-focal hepatocyte DNA synthesis. These subchronic effects are similar to those produced by unleaded gasoline. However, MTBE did not significantly increase the mean size of hepatic foci and volume fraction of the liver occupied by foci as compared to *N*-nitrosodiethylamine-initiated controls at either 16 or 32 weeks. The lack of tumor-promoting ability of MTBE in *N*-nitrosodiethylamine-initiated

female mouse liver was unexpected, and suggests that MTBE does not produce liver tumors through a tumor-promoting mechanism similar to that of unleaded gasoline.

Because MTBE is metabolized by cytochrome P450 to formaldehyde, a potentially mutagenic intermediary capable of forming DNA-protein cross-links, the formation of DNA-protein cross-links and of another formaldehyde derivative, RNA-formaldehyde adducts, from MTBE was investigated using freshly isolated hepatocytes from female CD-1 mice incubated with MTBE (Cassanova and Heck 1997). DNA-protein cross-links and RNA-formaldehyde adducts were detected, but adduct yields were very modest and independent of the concentration of MTBE in the hepatocyte suspension over a wide range of concentrations (0.33 - 6.75 mM). Similar results were observed using hepatocytes from male $B6C3F_1$ mice and male Fischer 344 rats. Induction of cytochrome P450 by pretreatment of mice with MTBE (1.8 g in corn oil/kg bw via gavage once daily for three consecutive days) prior to the isolation of hepatocytes did not result in a measurable increase in the yields of either DNA-protein cross-links or RNA-formaldehyde adducts. In contrast to the absence of concentration-dependent DNA-protein cross-link and RNA-formaldehyde adduct formation from MTBE, there was a marked, concentration-dependant increase in the yields of both DNA-protein cross-links and RNA-formaldehyde adducts when formaldehyde was added directly to the medium. These results suggest that the metabolism of MTBE and formaldehyde approach saturation at concentrations below 0.33 mM, and that the rate of formaldehyde production from the metabolism of MTBE is slow relative to the rate of formaldehyde metabolism. The findings of Cassanova and Heck (1997) clearly demonstrated that, although formaldehyde is a minor metabolite of MTBE in rodents, it is unlikely to be a mutagen *in vivo* as a result of ambient exposures to MTBE because (1) the concentrations of formaldehyde from MTBE were very small; (2) the absence of concentration-dependent DNA-protein cross-links and of RNA-formaldehyde adducts—each a biomarker of biologically reactive formaldehyde; and (3) the rate of formaldehyde is sufficiently slow to preclude reaching saturation of formaldehyde and to assure that formaldehyde from MTBE is detoxified before it might react with genetic macromolecules. Therefore, the metabolism of MTBE to formaldehyde is not a critical component of MTBE's carcinogenic mechanism in mice and most likely rats.

Bird *et al.* (1997) hypothesized another possible mechanism to explain the liver adenomas in female mice: an MTBE-mediated change in the homeostatic balance of estrogen in liver. Bird *et al.* noted that, at the 3000 and 8000 ppm exposure levels, endometrial cell hyperplasia was decreased in the uterus. Because estrogens are principally responsible for maintaining the endometrial cell state of proliferation and are known to suppress mouse liver tumor promotion, high concentrations of MTBE may change the homeostatic balance of estrogen resulting in liver tumor promotion as a secondary effect.

This hypothesis was reiterated by Mennear (1997) who noted that the increase in hepatocellular adenomas after high doses of MTBE may be a sex-specific effect. Although a mechanism to explain the effect is not readily apparent, Mennear suggests a hormonally-mediated effect is likely since only females were affected. In this regard, estrogens are known to suppress the promotion of liver tumors in mice (Lee *et al.* 1989). Therefore, decreased circulating estrogens might promote the development of liver tumors.

Furthermore, HEI (1996) has stated that hepatocarcinogenesis in mice (spontaneous and induced) has been shown to be under genetic control of "risk modifier" genes and under strong hormonal control. HEI cited as an example that castrating male mice results in a decrease in liver tumors, even though ovariectomies in female mice cause an increase in hepatic tumors. These hormonal influences are thought to be mediated by hormones controlling the proliferation of pre-neoplastic foci. It is conceivable that exposing mice to high concentrations of MTBE for a prolonged time causes imbalances in the hormonal homeostasis and thus influences the development of pre-neoplastic and neoplastic liver foci.

Therefore, mounting evidence strongly suggests that the female mouse liver tumors are the result of high non-genotoxic doses of MTBE interfering with the tumor suppressor mechanisms of estrogen in female mouse liver—mechanisms that are not known to be present in human liver (Vesselinovitch, 1990).

4. Evidence suggests that the testicular tumors in rats were likely to result from factors other that MTBE.

MTBE toxic doses (inhaled and by gavage) were associated with testicular tumors in rats and not in mice similarly exposed (Bird *et al.* 1997; Belpoggi *et al.* 1995). The relevance of these responses to humans is seriously questioned. As noted by Bird *et al.* (1997), this tumor is "typically the most frequently observed spontaneous tumor" in aged Fischer 344 rats. In addition, Bird *et al.* concluded that the lower than expected incidence rate for testicular tumors in the control group suggested that these findings may not be treatment related. The incidence observed in the MTBE exposed groups were within the range previously reported for aged male Fischer 344 rats.

The inherent flaws of the Belpoggi *et al.* (1995) gavage study (see below) preclude drawing any causal inference between any exposure to MTBE and the Leydig cell testicular (or any other) tumors. A committee of the National Academy of Sciences (NRC, 1996) questioned the applicability of the Leydig cell testicular tumors by noting "high-dose male rats showed increased survival after 88 weeks...and therefore were at greater risk for development of these late-appearing neoplasms(first tumor was found at 96 weeks). It is unclear whether the

statistical analysis (presented by Belpoggi *et al.*) adjusted for this." Therefore, the evidence suggests that the testicular tumors reported in rats were likely to result from factors other that MTBE, and are not relevant for humans exposed to ambient levels of MTBE.

5. The findings of the gavage study are considered sufficiently flawed and provide no evidence for judging whether MTBE might be reasonably anticipated to be a human carcinogen.

In the study by Belpoggi *et al* (1995), MTBE administered to rats via gavage was reported to have produced Leydig cell tumors, lymphomas, and leukemias. Numerous flaws with this study limit its applicability for humans and in estimating the cancer potency of MTBE. The National Academy of Sciences (NRC, 1997) observed that the study by Bird *et al.* (1996) did not report any lymphomas or leukemias in either sex of the Fischer 344 rats even though the high dose tested (8000 ppm) was at least **four times greater** than the largest gavage dose, which was clearly toxic. This discrepancy is most noteworthy in light of the fact that Fischer 344 rats are highly prone to the induction of leukemia. NRC was also troubled by the fact that the increased incidence of lymphomas and leukemias was observed only at doses which clearly decreased survival. Further, lymphomas or leukemias were not elevated in males whose survival rate was not as severely impacted. In addition, the reduced survival rates indicated to NRC that the maximum tolerated dose was probably exceeded.

The Health Effects Institute (HEI), with its separate committee of experts, noted that the study by Belpoggi *et al.* (1995) is limited in its applicability in part because the investigators neglected to identify the type of lymphohematopoietic tumors observed and because they analyzed both lymphomas and leukemias as a single neoplastic lesion rather separately (HEI 1996). Had the investigators evaluated the leukemias and lymphomas separately, as is specified in the NTP guidelines, the association between MTBE and either lymphoma or leukemia would not have been statistically significant (Mennear, 1996). Suspicion that these findings are likely to be invalid is also drawn from the observation that rats subchronically and chronically exposed to doses of MTBE as high as those employed in this gavage study consistently develop nephropathy; however, none of the treated rats in this study were found to have nephropathy. Therefore, the findings of the gavage study by Belpoggi *et al.* (1995) are considered sufficiently flawed and provide no evidence for judging whether MTBE might be reasonably anticipated to be a human carcinogen via ingestion.

6. The overall data strongly supports the conclusion that MTBE is not reasonably anticipated to be a human carcinogen.

MTBE has been tested extensively to determine whether it can cause cancer and, if so, whether the findings are relevant for humans exposed to ambient levels of this compound. Although toxic doses of MTBE have caused cancer in laboratory rats and mice, the preponderance of evidence indicates that it is reasonably anticipated to not be a human carcinogen. This conclusion is based on the observations that

(1) MTBE is not a genotoxicant and is highly unlikely to be a genotoxic carcinogen because it has been shown repeatedly in numerous genotoxicity tests to cause no genetic alterations. In the one test that was positive, research findings demonstrated compellingly that the metabolite, formaldehyde, was unlikely to be present at cellular concentrations sufficient to cause macro-molecular changes that might lead to a carcinogenic response.

(2) The pattern of kidney tumors in male rats and the results of extensive mechanistic studies clearly indicate that this tumor type is specific to the male rat exposed to MTBE at toxic doses, and that this tumor type is not relevant to humans exposed to MTBE. Of particular note, the NRC committee stated that

> "the male rat kidney-tumor data probably should not be used for (estimating the cancer potency of MTBE) in light of the new information on its probable causation, i.e. α_{2u}-globulin nephropathy, which is thought to be unique to the male rat and **not relevant to humans** (emphasis added)."

(3) The pattern of liver tumors in the female mouse and the results of mechanistic studies suggest that this tumor type is specific to the female mouse exposed to MTBE at toxic doses, and that this tumor type is not relevant to humans exposed to MTBE.

(4) Based on consensus of the pathology community, the testicular tumors in rats are age-related and not compound-induced. Therefore, this tumor is not relevant to humans exposed to MTBE.

(5) The findings of the gavage study are unreliable indicators of the ability of MTBE to predict any carcinogenic potential for humans, because of the many methodological flaws in the design and execution of the study. Therefore, this study should not be used in judging whether MTBE should be listed by NTP in the next edition of its Report on Carcinogens.

These five conclusions are widely supported by several in depth evaluations by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1997),

the U.S. Health Effects Institute (HEI, 1996), and the National Academy of Sciences (NRC, 1996).

Particularly noteworthy is a conclusion from the international study by ECETOC:

> "Overall (the current MTBE database) demonstrates that MTBE has some potential to increase the occurrence of certain tumours in female mice or male rats after chronic, high-dose inhalation exposure. It is probable that these effects are secondary to very high-dose-induced toxicity, occur through a species-specific, non-genotoxic process and are therefore not predictive of a hazard to humans under normal exposure conditions." (ECETOC 1997)

A final consideration is that of human exposure. As noted in Brown (1997), the arithmetic mean for residential exposures to MTBE by inhalation were estimated to be between from 0.4 and 0.6 μg/kg-day. Such doses are considered to be well below doses that might cause any form of toxicity.

Therefore, the overall data strongly support the conclusion that MTBE is not reasonably anticipated to be a human carcinogen.

4 Summary and Conclusions

According to NTP's listing and delisting criteria, a substance can be listed either as a "known human carcinogen" or as "reasonably anticipated to be a human carcinogen") in its Biennial Report on Carcinogens (NTP, 1996).

4.1 **MTBE does not meet any criteria for the category "known human carcinogen"** because no human data have reported cancers associated with MTBE manufacture or use, despite it having been used for two decades.

4.2 **MTBE does not meet any criteria for the category "reasonably anticipated to be a human carcinogen"** for the following reasons:

> *Criterion # 1*: "sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites; (2) by multiple routes of exposure; or (3) to an unusual degree with regard to incidence, site, or type of tumor or age at onset."
>
> *As modified by the following*: "NTP acknowledges that applying these criteria requires "scientific judgment, with consideration given to all relevant information." Such relevant information includes, according to NTP, dose response, metabolism, pharmacokinetics, sensitive sub-populations, genetic effects, and other data relating to mechanism of action or factors that may unique to a given substance."

MTBE has elicited tumors in male rats and female mice; however, the relevance of these findings for humans are strongly mitigated by a substantial understanding of mechanisms of carcinogenicity and by other pertinent considerations:

- MTBE is not genotoxic, and, therefore, is not likely to be a genotoxic carcinogen.
- Compelling evidence exists to indicate that the male rat kidney tumors are likely to result from secondary, non-genotoxic tissue damage caused by otherwise highly toxic doses of MTBE; and such a mechanism is unlikely to exist in humans.

- Mounting evidence strongly suggests that the female mouse liver tumors are the result of high non-genotoxic doses of MTBE interfering with the tumor suppressor mechanisms of estrogen in female mouse liver—mechanisms that are not present in human liver.

- Evidence suggests that the testicular tumors in rats were likely to result from factors other than MTBE.

- The findings of the gavage study are considered sufficiently flawed to provide no evidence for judging whether MTBE might be reasonably anticipated to be a human carcinogen.

or

> *Criterion # 2*: "There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well defined structurally-related class of substances whose members are listed in a previous Annual or Biennial Report on Carcinogens as either a known to be human carcinogen or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans."
>
> MTBE has not been listed in previous editions of the NTP Report on Carcinogens. No evidence exists from other compounds to suggest, through structure-activity analysis, that MTBE might be capable of causing cancer in humans.

Therefore, "*sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors*" does **not** exist for MTBE, and the data reported herein strongly support the conclusion that **MTBE should not reasonably be anticipated to be a human carcinogen.**

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